Cancer Investigation, 23:700–711, 2005

ISSN: 0735-7907 print / 1532-4192 online
DOI: 10.1080/07357900500360008

Taylor & Francis
Taylor & Francis Group

ENVIRONMENTAL CARCINOGENESIS

Pesticides and Human Cancers

Michael C. R. Alavanja, Ph.D. and Matthew R. Bonner, Ph.D.

Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Rockville, Maryland, USA

The potential for human carcinogenicity of almost all pesticides currently on the market has been poorly evaluated and is inadequately understood. Generating mechanistic data in both animal studies and epidemiology will play an increasingly important role in the future. Improved exposure assessment, in large prospective studies that generate reliable exposure-response data that focus on individual pesticide exposures are needed. One of the greatest opportunities to make more rapid progress will be to foster more multi-disciplinary collaborations between toxicologists and epidemiologists. Collaborations on molecular epidemiology investigations offers such opportunities to both toxicologists and epidemiologists that were not possible even a decade ago.

Keywords Pesticides; Cancer; Multi-Disciplinary Studies; Toxicology; Molecular Epidemiology

DO PESTICIDES CAUSE CANCER?

Pesticides are "substances used to prevent, destroy, repel or mitigate any pest ranging from insects, animals and weeds to microorganisms. . . . [1] Pesticides are pervasive in our environment. In 1999, over one billion pounds of pesticides were applied in the United States and over 5.6 billion pounds were applied worldwide.[1] Some pesticides, including the organochlorines (e.g., aldrin, chlordane, DDT, dieldrin), lead arsenate, creosote, and sulfallate, are carcinogenic in animals[2–4] and are no longer registered for use in western nations. Many of these pesticides, however, continue to be used in developing nations.[5] Although the acute toxicity of pesticides generally is well characterized by animal testing (and human experience), the potential for human carcinogenicity of almost all compounds currently on the market has been poorly evaluated and is inadequately understood despite 50 years of epidemiologic and toxicologic research. The International Agency for Research on Cancer (IARC) has classified "occupational exposures in spraying and application of non-arsenical insecticides" as a group as "probable human carcinogens" (category 2A).[4] Although, other than the arsenical insecticides and TCDD (a contaminant of the phenoxy herbicide 2,4,5-T), which are identified as human carcinogens by IARC (category 1), the epidemiological evidence for all other compounds is weak and inconclusive. Without identifying whether a specific compound is responsible for the cancer link, appropriate and effective public health measures are difficult to establish. In this review, we will evaluate the strengths and limitations of current scientific evidence linking pesticides and cancer and discuss strategies to fill in the gaps in our current understanding.

Address correspondence to Michael C.R. Alavanja, Occupational and Environmental Epidemiology Branch, National Cancer Institute, NIH, DHHS, 6120 Executive Blvd. EPS 8000, MSC 7240, Bethesda, MD 20892-7240. E-mail: alavanjm@mail.nih.gov

ESTABLISHING A PRIOR HYPOTHESES FOR EPIDEMIOLOGY STUDIES OF HUMAN CANCER: STANDARD CARCINOGENIC BIOASSAYS

The standard bioassay to determine the human carcinogenic potential for chemical agents is the two-year feeding studies of rodents (rats or mice). This type of assay, however, is very costly and time intensive.[6] Given that there are 890 registered active ingredients and thousands of pesticide formulations registered for use in the United States (USEPA sales and usage, 1996 and 1997), it is impractical to conduct rodent feeding studies on all of these pesticides. Even when a pesticide is tested, interpreting the results and making crossspecies extrapolations and extrapolations from very high doses to low doses are fraught with uncertainty. Further, the administered doses, while generally not high enough to cause overt toxicity, may damage the epithelium of various organs thus stimulating a mitogenic response by the injured epithelium. It may be this mitogenic response is due to cytotoxicity (which is unlikely to take place at exposure levels usually experienced by humans) rather than a carcinogenic effect of the agent driving cell proliferation that may greatly increase the probability of developing cancer in animal bioassays.[7,8] In addition, the mode of action cannot be identified by these feeding studies, providing little information that generally is considered necessary to extrapolate carcinogenic risk to humans.[6]

In lieu of these long-term assays, short-term bioassays have been developed based on our understanding that genotoxicity plays a major role in the induction of carcinogenesis by many agents.[9] The short-term bioassays have become the standard

methods used to assess the carcinogenicity of chemicals[6,10] including pesticides. Commonly used short-term assays generally include a bacterial assay (i.e., Ames test), a mammalian cell assay (i.e., mouse lymphoma assay) and a cytogenetic assay assessing structural and numerical chromosomal aberrations.[6,9] Extrapolations from short-term bioassays also are difficult and the predictive ability of these assays has been disputed,[6,9,11–16] in part because they fail to account for metabolic differences between species.[9]

The sensitivity of the Ames *Salmonella* mutagenicity assay to identify mutagens as carcinogens has been estimated at 45–90 percent.[14,16–18] With regard to the other short-term bioassays, the sensitivities and specificities suggest that these assays (chromosome aberration: sensitivity = 55 percent; specificity = 69 percent, sister chormatid exchange: sensitivity = 73 percent; specificity = 45 percent, mouse lymphoma: sensitivity = 70 percent; specificity = 45 percent) separately or in combination may not be sufficient to predict the carcinogenicity of an agent in the rodent model,[14] let alone predict human carcinogenicity. Shelby[19] found the *Salmonella* assay had a sensitivity of 17 (77 percent) for 23 IARC Group 1 human carciogens, while Wilbourn et al.[15] concluded that the rodent assays were predictive for 84 percent of 44 known or suspected IARC carcinogens (not that much higher than the *Salmonella* bioassay sensitivity reported by Shelby).

Despite these limitations, long-term and short-term assays often are used to guide epidemiologic efforts in determining which agents to investigate. We now recognize that some agents may promote carcinogenesis through nongenotoxic mechanisms by inducing noncytotoxic cellular proliferation or by inducing peroxisome proliferation that may lead to the generation of reactive oxygen species that in turn can damage DNA.[20] Other potential mechanisms for nongenotoxic carcinogens include modulation of signal transduction pathways and inhibition of gap junctional intracellular communication.[21] and immunotoxicity.[22]

New bioassays, such as transgenic mouse models, have incorporated advances made in our understanding of carcinogenesis and may prove to be much more sensitive and specific in detecting the potential human carcinogenicity of an agent than the older bioassays,[10,23] although our experience in using these newer bioassays is limited to a fraction of the pesticides on the market.

EVALUATE PESTICIDE EPIDEMIOLOGY IN THE ABSENCES OF A PRIORI HYPOTHESES

Since the entire battery of current long-term and short-term bioassays is not available for all pesticides and since the sensitivity and specificity of most long and short-term bioassays are well below 90 percent, establishing firm a priori hypotheses about human carcinogenicity frequently is not possible. In the absence of a strong a priori hypothesis linking a specific pesticide to a specific cancer, observations from classical epidemiology showing a positive association are sometimes called into question. Critics may label the results false positives, typically citing one of the following methodological flaws: 1) The association occurred by chance alone, since the study subjects had multiple exposures and the evaluations made multiple comparisons; 2) the information on exposure was inadequate to distinguish varying degrees of exposure, so exposure-response evaluation was impossible; 3) the information on exposure was collected after the cancer developed (in case-control studies), resulting in a case-recall bias and a false positive result; 4) information on lifestyle and other occupational exposures was lacking and it was these 'other factors' that are statistically associated with the pesticide exposure, and not the pesticide that is the true carcinogen (i.e., confounding bias); 5) the biological basis for the association was unknown, the animal data were negative, and so we must disregard the epidemiological result.

Conversely, other critics have argued it is even more likely that some pesticides are actually unrecognized human carcinogens.[24] Failure to recognize these compounds as carcinogens may be due to the inadequacy of exposure assessment, sample size, and study design. Analytic epidemiology of pesticides needs to progress beyond studying pesticides in aggregate and begin to study the health effects of exposure to individual pesticides. Conventional classifications of pesticides (i.e., insecticide, herbicide, and fungicide) are not made with regards to the mode of carcinogenic action and, therefore, studies based on aggregate classification likely are to obscure associations with individual pesticides. Similarly, positive associations in studies with few exposed cases inevitably result in tentative conclusions and few specific public health recommendations.

CONSOLIDATED REVIEW OF EPIDEMIOLOGICAL STUDIES

Commercial pesticides usually are not a single active ingredient, but include a mixture or solution of both "active ingredients" and "other ingredients." The health effects of a pesticide product may result from exposure to either the active ingredient or the other ingredients in the formulation or both. While the active ingredient is public information, "other ingredients" may include a wide array of compounds and information about these ingredients often is considered confidential business information and is not publicly available. Some evidence from animal testing suggests the commercial formulation of a pesticide may cause greater biological effect on the animal then does the pure active ingredient.[25–29] For example, the commercial product "Roundup" [containing the active ingredient glyphospate] was associated with increased DNA adducts in mice[26] and a weak mutagenic effect in the *Salmonella* assay,[27–29] whereas glyphosate alone did not show these effects.

Our review of the literature has been organized by chemical class of the "active ingredient" and the conclusions reached by the authors cited usually refer to the "active ingredient" or the chemical class of the "active ingredient," but the reader is advised that the exposure is actually to a complex formulation of compounds. The chemical structures of a sample of these pesticides are shown in Figure 1.

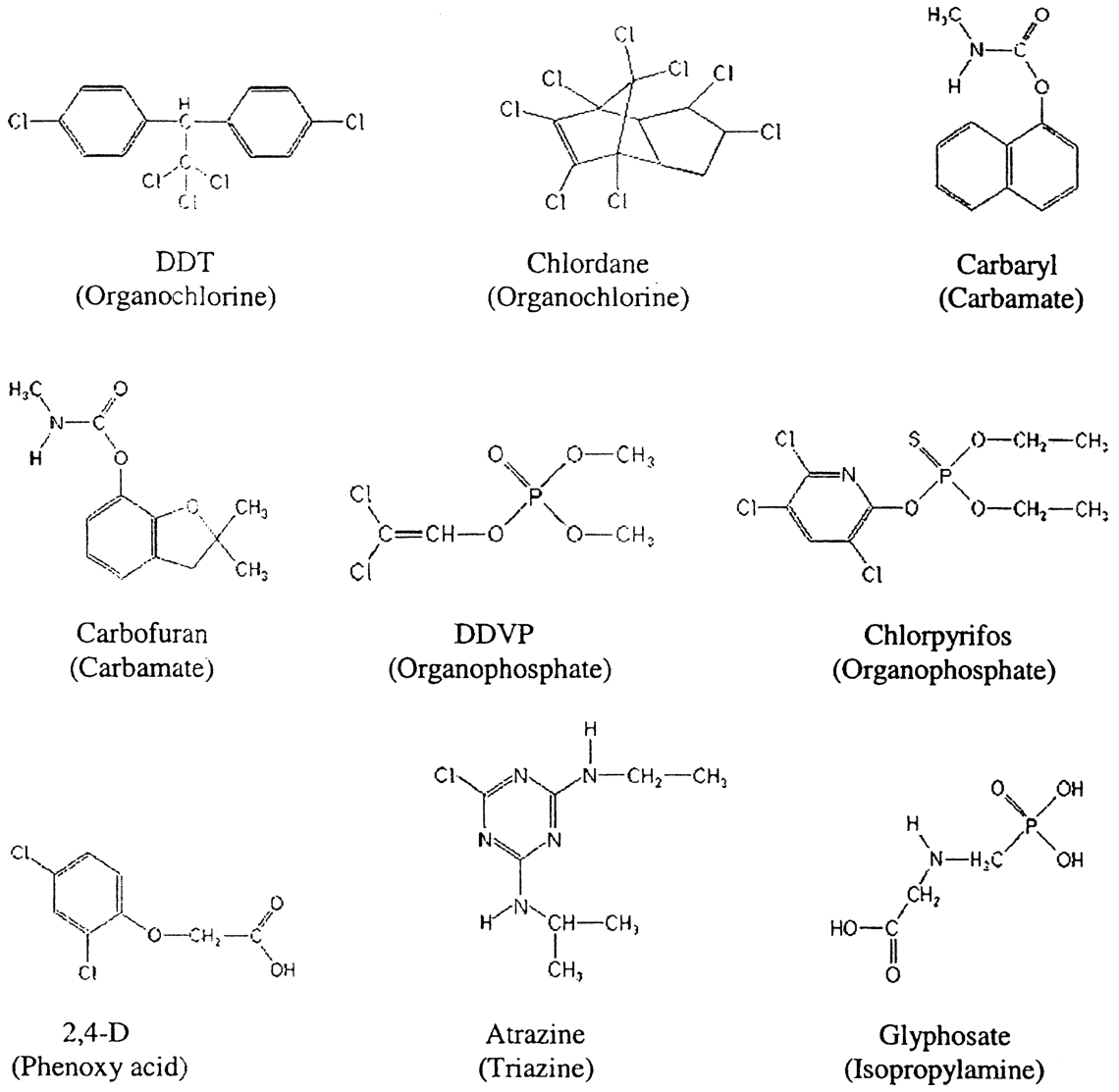

FIG. 1. Chemical structure of selected pesticides.

Organochlorine Insecticides

Organochlorine insecticides belong to three chemical classes: 1) dichlorodiphenylethanes, 2) cyclodienes, and 3) chlorinated benzenes and cyclohexanes.[30] Collectively, organocholorine insecticides are very effective because they have low volatility, high chemical stability, high lipid solubility, and slow biotransformation and degradation. These same characteristics, however, make these insecticides particularly hazardous to non-pest organisms (flora and fauna), including humans.

In 1991, IARC (monograph 53) reviewed chlordane and DDT for carcinogenicity and concluded that they were possibly carcinogenic to humans (Class 2B).[4] In particular, IARC specified that there was sufficient evidence from experimental animals for carcinogenicity, but that there was inadequate evidence for human carcinogenicity. The genotoxicity bioassays reviewed by IARC suggested that DDT did not induce DNA damage in bacteria, yeast, or cultured mammalian cells, including cells harvested from human. The results from chromosomal aberration assays were mixed. In long-term feeding studies, the incidence of liver tumors and leukemia was elevated in exposed rodents. Chlordane also was shown not to damage DNA. It was clastogenic, however, and increased the incidence of malignant tumors in rodents.

Organochlorine insecticides have been associated with non-Hodgkin lymphoma (NHL), brain cancer and other cancers of the central nervous system, prostate cancer, pancreatic cancer, breast cancer, and liver cancer.[4] However, exposure assessments generally have been weak and the studies have not linked specific pesticides to cancers in a consistent fashion.

Studies using biologic measures of organochlorine pesticides have been inconsistent. Hardell et al.[31] found an NHL risk to be associated with serum chlordane and related compounds collected postdiagnostically, but in a population-based, case-control study using prediagnostoc serum levels of several

organochlorine compounds, Cantor et al.[32] could not confirm this finding. The inconsistency between these two studies may illustrate the need for prediagnostic biological samples to establish etiological associations.

The estrogenic activity of DDT, DDE, and other chlorinated pesticides has been hypothesized to increase the risk of breast cancer, but numerous cohort and case-control studies failed to show a convincing association.[33–44]

The great majority of cohort studies of pesticide workers have not indicated an excess risk of brain and central nervous system cancer associated with organochlorine.[45] Although, higher levels of organochlorine compounds were found in adipose tissue of brain cancer patients than noncancer patients, but studies have failed to identify specific pesticides.[46]

A majority of epidemiologic studies have demonstrated an association between prostate cancer and farming and/or pesticide exposure, but the relative risk estimates were generally less than 1.5 (50 percent excess risk). The specific pesticides responsible have not been identified, but a number of investigations have suggested an association with organochlorine insecticides as well as other pesticides.[46–53]

Pancreatic cancer risk was elevated in a number of occupational studies of agricultural workers and pesticide users, including farmers,[54–56] and licensed and unlicensed agricultural pesticide applicators.[57–62] Garabrandt et al.[63] observed elevated risk of pancreatic cancer among DDT manufacturing workers (OR = 4.8, 95% CI 1.3–17.6). Another study from Australia showed a five-fold increased risk associated with DDT application.[64] Two published reports examined the role of DDT and mutation of K-ras, a growth signal transduction gene that commonly is mutated in pancreatic cancer tumors,[65,66] but these studies were inconsistent.

Liver cancer has been associated with DDE levels in adipose tissue in Whites but not among African-Americans.[67] While some studies have suggested that farmer laborers have elevated risk of liver cancer, other studies have not demonstrated such an association.[68]

It is curious that epidemiologic studies to date have been unable to provide clear evidence to either support or refute the conclusions regarding human carcinogenicity of pesticides based on the animal data.[69] One possible explanation for this paradox may be the exposure assessment in the epidemiologic studies was inadequate to detect the carcinogenic effects of DDT or chlordane exposure. Conversely, the epidemiologic evidence may have identified correctly the association and the animal evidence was spurious because of the inherent limitations of bioassays.[69] Regardless, situations likes these emphasize how difficult and complex the process of causal inference can be when it comes to determining etiologic factors for disease.

Carbamate Insecticides

Carbamate insecticides are a group of compounds with a carbamate group that binds to acetylcholine esterase to elicit neurotoxicity. Acetylcholine esterase hydrolyzes acetylcholine, an important neurotransmitter. During acute poisoning with these compounds, the concentraion of acetylcholine is increased in neuronal synapses leading to overstimulation of cholinergic receptors. Carbamate insecticides are considered reversible inhibitors of acetylcholine esterase because they readily disassociate with acetylcholine esterase and generally are considered less hazardous than organochlorine and organophosphate pesticides. Potential carcinogenic modes of action currently are unknown.

In a nested case-control study of Florida structural pest control workers, those who used carbamate insecticides were observed to have an excess lung cancer risk (OR = 16.3; 95%CI = 2.2–122.5), although no specific carbamate insecticides were associated the excess risk.[70] A similar excess risk for lung cancer was not observed in a population-based case-control study among residences of Saskatchewan, Canada.[71] NHL was observed to increase among those occupationally exposed to carbamate pesticides (OR = 1.9, 95%CI = 1.2–3.0) in the "cross-Canada study of pesticides and health"[72] and carbofuran, a carbamate insecticide, was associated with a significant elevation in NHL risk among farmers using this pesticide (OR = 1.6, 95%CI = 1.1–2.3).

The USEPA classified carbaryl, a carbamate insecticide, as "likely to be carcinogenic to humans" primarily because exposure increased the incidence of vascular tumors in mice compared with nonexposed control mice.[2] In addition, carbaryl has been shown to induce as well as promote malignant tumors when applied topically to Swiss albino mice.[73] Sister chromatid exchanges also were observed in V79 hamster cells treated with carbaryl.[74]

Epidemiologically, carbaryl was found to be a risk factor for NHL in a pooled analysis of three population-based, case-control studies in the U.S. Midwest.[75] Carbamate insecticides, along with several other pesticide classes, also were found to be a risk factor for NHL in a Canadian multicenter population-based, case-control study.[72]

Carbofuran is a widely used carbamate insecticide registered for use on corn, alfalfa, and tobacco.[76] Laboratory data have shown that carbofuran is weakly mutagenic in some strains of *S. typhimurium*,[77] but *N*-nitosocarbofuran, derived from nitrosation of carbofuran, was more consistently was shown to have mutagenic properties.[78] Carbofuran was not associated with an increase in the incidence of malignant tumors in several two-year rat feeding studies[79] and the USEPA currently has listed carbofuran as "not likely to be carcinogenic in humans."[2]

The epidemiological data on the human carcinogenicity of carbofuran is inconclusive. Farmers who used carbofuran had elevated risk of NHL in at least two studies (NHL) (OR = 1.6; 95%CI = 1.1, 2.3) and NHL (OR = 1.6; 95%CI = 0.7–3.9).[72,75] A small, nonsignificant elevation in risk was observed among 21 exposed NHL cases in the Agricultural Health Study (AHS), a large prospective cohort study with comprehensive exposure information collected prior to onset of cancer for 50 important agricultural chemicals, including carbofuran. The rate ratios for NHL increased with increasing lifetime exposure-days of carbofuran use (OR = 1.0 [referent = 0 days], 0.8 (<9 days), 1.3 (9–39 days), 1.4 (>39 days)); although the

p for trend was not statistically significant (p trend = 0.40).[80] Pesticide applicators who applied carbofuran were also at an elevated risk of lung cancer with increasing use (OR = 1.0 [referent = <24.5 days], 1.4 (24.5–108.5 days), 2.3 (>108.5 days) p for trend = 0.05) in the AHS.[81] While the nonsignificant increasing trend of NHL with increasing days of carbofuran use and the borderline significant trend for lung cancer risk with increasing days of carbofuran use do not, by themselves, demonstrate a causal relationship, they suggest that carbofuran may be a human carcinogen. Further follow-up of the AHS cohort should clarify this picture.

Should carbofuran prove to be a human carcinogen, the mechanism of action will need to be studied since conclusive data are lacking. The fact that nitrosation of carbofuran produces a mutagenic intermediate (*N*-nitrosocarbofuan) is of interest,[78] as is the observation that carbamate insecticides impair immunity in animals[82] and humans.[83]

Organophosphate Insecticides

Organophosphorous (OPs) compounds are a large and diverse family of chemicals. The nomenclature is complex and the classification may follow various schemes. The large majority of OPs insecticides may be regarded as derivatives of phosphoric acid (e.g., DDVP). Large subclasses of OPs are the sulfur-containing compounds (phosphorthionates), which include parathion, diazinon, chlorpyrifos, and many others. Other important classes are the phosphorothiolates (demeton II, omethoate), phosphorothiolates (phorate, malathion), and phosphordithiolates (ethoprop, terbufos). The acute lethality of OP insecticides as a class of compounds largely is due to their ability to inhibit acetylcholinesterase.

In a Canadian multicenter population-based, case-control study among men with a diversity of occupations, McDuffie et al.[72] found that among major chemical classes, the risk of NHL was statistical associated with increased exposure to organophosphate insecticides and other herbicides and insecticides.

Chlorpyrifos, one of the most widely applied organophosphate insecticides (phosphorthionates) in the United States,[1] used to control pests on a variety of food crops, turf, ornamental plants, greenhouses, sod, indoor pest control, structural pest control, and pet collars.[84] Laboratory data report that chlorpyrifos induces mutagenicity.[85,86] sister chromatid exchanges[87,88] and chromosomal loss.[89]

Prior to the AHS the epidemiological literature provided little support for the human carcinogenicity of chlorpyrifos and the latest evaluation by the USEPA has classified chlorpyrifos as a Group E (i.e., evidence of noncarcinogenicity for humans).[2] Recently published data from the AHS, however, found a significant exposure-response pattern for chlorpyrifos with the risk of lung cancer increasing as the number of lifetime exposure days increased (p for trend = 0.036) while controlling for the effect of smoking and other know lung cancer risk factors.[90]

Should chlorpyrifos prove to be a human carcinogen, the mechanism of action is not known, but it is known that chlorpyrifos is metabolically activated in the liver to the active metabolite, chlorpyrifos oxon, which produces neurotoxicity by inhibiting esterases in the peripheral and central nervous system.[91,92] In studies among rats, chlorpyrifos induced mitotic abnormalities and cytotoxicity,[93] immunologic abnormalities,[94] generation of reactive oxygen species, DNA damage, and lactic acid dehydrogenase leakage.[95] In humans, increased CD26 expression and frequency of antibodies were found.[96] In other experimental studies, chlorpyrifos modified endogenous antioxidants, superoxide dismutase, glutathione peroxidase, and glutathione, which may lead to the development of oxidative stress as well as and also decrease activity of glutathione-*S*-transferase, an important metabolic enzyme responsible for detoxification of numerous xenobiotic compounds.[97]

Phenoxy Acid Herbicides

Phenoxy acid herbicides are a group of compounds with a phenoxy acid group used extensively in the corn belt and for small grain production as pre- and postemergent herbicides. Some of these compounds such as 2,4 D also have widespread use on lawns and gardens for weed control. 2,4,5 T, a phenoxy acid herbicide widely used in the 1960s, was found to be contaminated with dioxin (i.e., TCDD a human carcinogen) until manufacturing processes changed in the mid-1960s, resulting in much lower TCDD concentrations.

In 1986, IARC reviewed the evidence for carcinogenicity of chlorophenoxy herbicides, including 2,4-D, and concluded that there was limited evidence for carcinogenicity to humans. More recent genotoxicity bioassays found no evidence that 2, 4-D was genotoxic.[98] Similarly, 2,4-D did not increase the formation of micronuclei in vivo.[99] Further, chronic feeding studies conducted in rodents found no increase in the incidence of malignant tumors.[100]

The analytical epidemiological literature linking phenoxy herbicides to cancers include both cohort and case control studies. Among the cohort studies, Becher et al.[101] found an SMR of 326 (CI 119–710) for NHL in a group of workers in phenoxy herbicide plants in Germany, the excess occurring in the groups with the highest exposure to dioxins and other contaminants of phenoxy acids. Hooiveld et al.[102] found an RR of 1.7 (95%CI = 0.2–16.5) with RR increasing parallel to serum levels of TCDD indicating an exposure-related risk. Lynge et al.[103] did not find an elevated risk of NHL among a group of Danish phenoxy herbicide factory workers exposed to the herbicide MCPA, nor did Asp et al.[104] find an increase risk among Finnish chlorphenoxy herbicide applicators. Both of these studies were relatively small and firm conclusions could not be reached. Morrison et al.[105] conducted a large study of 155,000 farmers in Canada and found a statistically significant increase in NHL (RR = 2.11, 95%CI = 1.1–1.9), but specific phenoxy acid herbicides responsible for the excess were not identified. Thorn

et al.[106] did not find elevated rates of NHL among Swedish lumberjacks exposed to phenoxy herbicides. Zahm[107] found a slight excess of NHL (SMR = 1.14, 95%CI = 0.31–2.91) among employees of a lawncare company that used phenoxy acid herbicides, but the cohort was small and young and associations with specific pesticides could not be determined at that time.

Among the case-control studies, Hardell and Ericksson[108] first found an elevated risk in males over 25 years old for exposure to herbicides (OR = 1.6, 95%CI = 1.0–25), fungicides (OR = 3.7, 95% CI = 1.1–132.0), and specifically, phenoxy acids (OR = 1.5, 95%CI = 0.9–2.4). In a second study, Hardell et al.[109] found significantly elevated ORs with exposure to herbicides, in general, as well as exposure to phenoxyacetic acids, glyphosate, and MCPA in particular. Kogevinas et al.[110] did not find an elevated risk in connection with several phenoxy herbicides, though small excess were found with TCDD. While Persson et al.[111] found an OR of 2.3 (95%CI = 0.7–7.2) for NHL with occupational exposure to phenoxy herbicides, though the exposure was not quantified.

Triazine Herbicides

Triazine herbicides are a group of compounds with a triazine group that are used widely pre- and postemergent herbicides. Frequently used triazine herbicides include atrazine, simazine, cyanazine, metribuzin. Atrazine is used primarily on corn and soybean crops to control for the growth of broadleaf and grassy weeds. An estimated 76.4 million pounds of atrazine are applied annually in the United States, making atrazine the highest use agricultural pesticide.[1]

Animal data have shown that atrazine is associated with increased incidence and early onset of mammary tumors in female Sprague-Dawley rats with oral administration.[112,113] Atrazine also was associated with lymphomas and testicular cancer in rats and mice,[114–116] but the animal data were somewhat inconsistent.[117] IARC has classified atrazine as "possibly carcinogenic to humans," a 2B human carcinogen,[117] while the USEPA has classified atrazine as "not likely to be a human carcinogen."[118]

A greater than expected numbers of cases of cancer of the prostate, bladder, and oral cavity, and of lymphomatopoietic cancers were observed in a cohort of triazine manufacturing workers; however, none of the increases was statistically significant and exposures other than atrazine also occurred.[119] In case-control studies conducted in the U.S. Midwest, atrazine or triazine use was not associated with Hodgkin's disease,[120] leukemia,[121] multiple myeloma,[122] soft tissue sarcoma,[120] or colon cancer.[123] However, in a pooled analysis by De Roos et al.[124] of data of these studies found increased odds ratios for NHL with atrazine exposure in combination with exposure to one of three other pesticides (diazinon, alachlor, and dicamba). A case-control study of ovarian cancer found an increased risk among women farmers "possibly" and "definitely" exposed to atrazine in their occupation.[125] Rusiecki et al.[126] found no clear association between atrazine and any cancer in the AHS cohort, but follow-up was recommended for tumor sites in which there was a suggestion of a trend (lung, bladder, NHL, and multiple myeloma).

Isopropylamine

Glyphosate [N- (phosphonomethyl)glycine], commonly sold in the commercial formulation Roundup (Monsanto Company, St. Louis, MO), is one of the most frequently used broad-spectrum herbicides in the world.[1,127] Some studies found slightly greater genotoxicity in the formulation Roundup compared to glyphosate alone.[128,129] Roundup also was found to increase DNA adducts in mice[26] and was weakly positive in a variety of mutagenicity assays,[27–29] but these effects were not observed with glyphosate alone. Chronic feeding studies of glyphosate have not provided evidence of a carcinogenic effect in mice or rats.[127] De Roos et al.,[130] found no association overall between glyphosate exposure and cancer incidence (all cancer combined) in the AHS, nor with most other cancers. However, there was a suggested association for multiple myeloma.

EVALUATING EPIDEMIOLOGICAL EVIDENCE LINKING SPECIFIC PESTICIDES TO CANCER IN THE ABSENCE OF FIRM A PRIORI HYPOTHESES

Pesticides are used by hundreds of millions of people in agricultural, commerce, public health, and for home and gardens purposes around the world. For many, pesticides are considered an important tool that is essential for their livelihood and welfare. Regulatory policy that prematurely identifies a pesticide as a carcinogen runs the risk of taking a relatively safe and valuable tool out of the pesticide-applicators toolbox. Conversely, when a pesticide is a human carcinogen, delayed action can put the pesticide-applicator and society at increased risk of serious disease.

Few epidemiological studies generate sufficient empirical evidence to establish, on their own, that a particular compound is a human carcinogen. A major problem with the evaluation of the carcinogenicity of pesticides in humans is that there are often no strong a priori hypotheses linking a specific pesticide with a specific cancer. As such, the findings from the most rigorous epidemiologic studies face the uncertain task of interpreting statistically significant exposure-response associations linking a specific pesticide to a specific cancer with little prior evidence supporting a link between a pesticide and outcome. This situation can lead to "false-positive associations." To guard against this type of error, the authors proposed a set of guidelines (Table 1) that might be used to interpret epidemiologic studies in this environment. Under these circumstances, it might be reasonable to expect that an evaluation of human carcinogenicity of a specific pesticide from a study or series of studies should replicate a significant positive exposure-response trend with a

TABLE 1
Potential guidelines for the evaluation of the epidemiological evidence linking specific pesticides to cancer

Level of evidence category	Conclusion	Strength of epidemiologic	Consistency of evidence	Recommended public health action
1	Human carcinogen	1. Significant positive exposure-response. 2. Documentation of internal exposure on a sample of study subjects. 3. Evidence of relevant biological effect in humans or animals.	1. Significant exposure-response in two geographic areas and/or two periods of time.	1. Immediate review of registration.
2	High level of evidence	1. Significant positive exposure-response. 2. Documentation of internal exposure on a sample of study subjects.	1. Significant exposure-response in one geographic area and one point in time.	1. Immediate review of registration.
3	Moderate level of evidence	1. Significant positive exposure-response.	1. Significant exposure-response in one geographic area and one point in time.	1. Early review of registration.
4	Modest level of evidence	1. Positive exposure-response association but not significant.	1. Non-significant exposure-response in one geographic area and one point in time.	1. Standard level of regulatory review.
5	Inadequate evidence	1. Positive evidence of association or evidence of no association, but nonsignificant and non-monotonic exposure-response pattern.	1. Positive evidence but non-significant exposure-response in one geographic area and one point in time.	1. Standard level of regulatory review.
6	Evidence of no effect	1. Sufficient statistical power to detect an excess relative risk (or odds ratio) of 20% in the highest exposure category. No evidence of any excess risk of cancer.	1. No evidence of exposure response in any geographic area or period of time.	1. Standard level of regulatory review.

specific cancer in two or more different geographic areas and/or at two or more different periods in time. Additional support for an association would also be generated if the population at risk can be shown to be exposed to the pesticide, its metabolite, or biomarkers of early effect in vivo. While the levels of evidence suggested by the authors in Table 1 are suggested merely as guidelines for interpreting evidence of human carcinogenicity, these or similar guidelines are particularly valuable when a priori hypotheses that link a chemical and a cancer are weak or inconclusive. If this degree of evidence was available (Category 1 or 2 in Table 1), it would be prudent for a regulatory body to immediately review and reconsider limiting use/or banning further use of the chemical. While other issues including economic impact should be considered, public health issues should predominate.

Since the biological mechanism of action of most known carcinogens is not understood completely, implementation of timely public health action could be needlessly delayed if we demand that the mode of action be understood before categorizing a pesticide as a human carcinogen. So, while it is not necessary to demonstrate a mode of carcinogenic action, whenever molecular epidemiologic studies demonstrate that the pesticide has a relevant biological effect, the weight of evidence supporting the declaration of a pesticide a carcinogen (see section below) is enhanced. Lesser degrees of epidemiologic evidence would indicate proportionally less immediate regulatory review (Table 1).

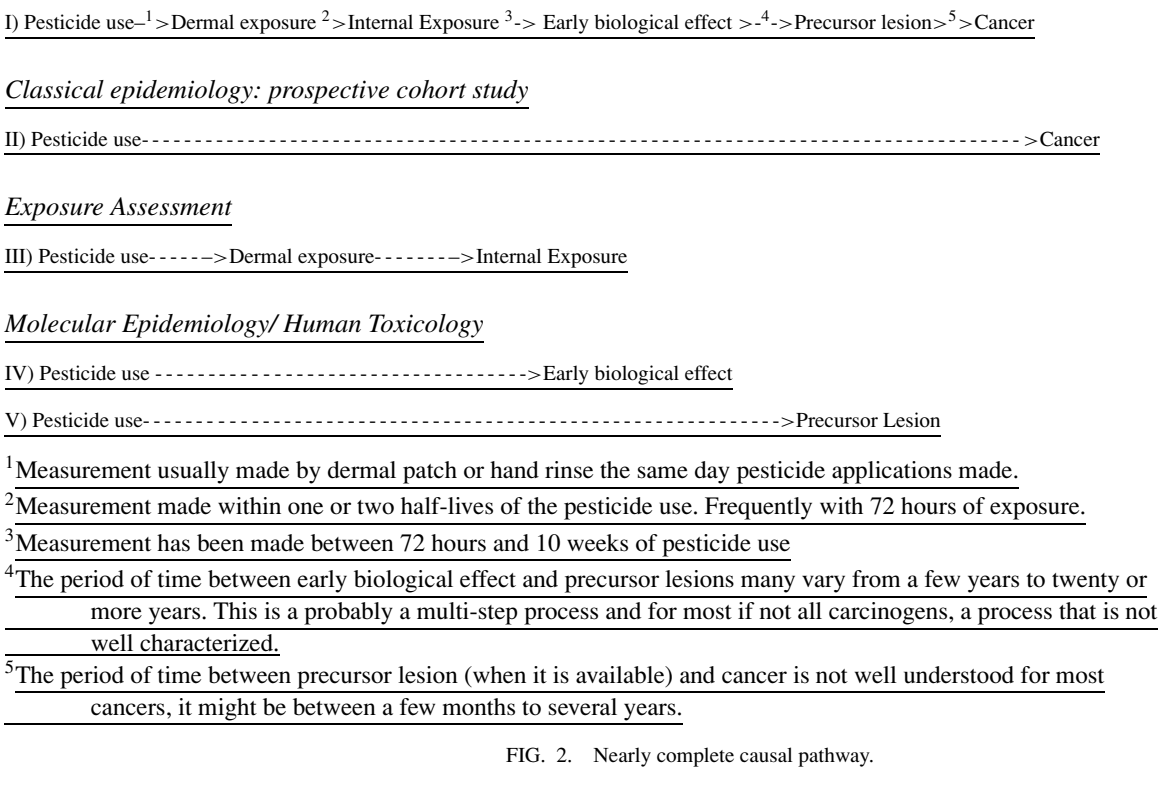

FIG. 2. Nearly complete causal pathway.

BIOLOGIC PLAUSIBILITY OF RESULTS FROM CLASSICAL EPIDEMIOLOGIC STUDIES

Cancer is a multistep and multifactor biological process that usually takes years to go from initial exposure through all the necessary intervening steps, finally resulting in cancer (Figure 2). Demonstrating all of these steps is rarely, if ever, possible in a single epidemiological study of cancer. Under the best circumstances, key steps along the causal pathway may be isolated (Figure 2 II-IV) in separate investigations and together they provide biological support for the classical epidemiology results (Figure 2, II) by demonstrating: internal exposure, some early biologic effect following exposure, identifying some persistent effects among those exposed, and by identifying some plausible mechanisms of action.

A multiplicity of biological pathways have been suggested by which pesticides may cause the cancers observed in excess among pesticide exposed populations, including genotoxicity,[131] perturbations in the immune and/or hormonal system.[132,133] To date, shortcomings in the investigations have limited establishing firm links between individual pesticide exposures and specific biological effects. One potential pathway that holds promise is the link between pesticide exposure and chromosome aberrations in peripheral blood lymphocytes.[134,135]

The frequency of cells with structural chromosome aberrations (CAs) in peripheral blood lymphocytes (PBLs) is the first genotoxicity biomarker that has actually shown an association with overall cancer risk[136–138] and recent data indicate that both DNA double-strand breaks and other initial DNA lesions responsible for chromosome-type and chromatid-type aberrations are associated with cancer risk.[139] The pooled study by Hagmar et al.[139] showed that the association between CA frequency and subsequent cancer incidence/mortality was not modified by sex, age, country, occupational carcinogenic exposure, or smoking habits. This suggests that a high CA level is predictive of increased cancer risk irrespective of the cause of the initial CA increase. The CA-related cancer risk did not change with time since the CA analysis, indicating that it does not reflect secondary effects of undetected cancer.[140] To date, this type of evidence has not been generated in studies of individual pesticides sufficient for causal interpretation.

CONCLUSION

In total, the results from both bioassays and epidemiology have yet to convincingly demonstrate the carcinogenic potential of most pesticides. Epidemiologic methods of exposure assessment and relatively small sample size of many studies may have obscured positive associations that do exist. Conversely, the epidemiologic studies finding no conclusive causal link may be correct and the animal evidence may be spurious because of the inherent limitations of bioassays.

Fortunately, the limitations inherent in epidemiological studies may be complemented by the strengths of human toxicological studies and vise versa. In epidemiology, a new generation of studies that focuses on increasing the precision of exposure

assessment of the agent under study and concomitant exposures and the collection of biological tissue, such as the AHS, will be complemented by toxicologic studies with an interest in human metabolic systems. Ideally, a hypothesis generated by either discipline should be coherent with regards to both epidemiological and toxicological observations. Therefore, these sciences, operating together, may be able to provide much greater insight into whether an agent is carcinogenic than either science can on its own. Clearly, one of the greatest opportunities to make more rapid progress will be to foster more multidisciplinary collaborations between toxicologists and epidemiologists. Fortunately, molecular epidemiology offers such an opportunity to coalesce toxicology and epidemiology in ways that were not possible a decade ago.

REFERENCES

1. Donaldson, D.; Kiely, T.; Grube, A. Pesticide industry sales and usage: 1998 and 1999 market estimates. USEPA, Washington, DC. 2002.
2. Department of Environmental Protection. Chemicals evaluated for carcinogenicity. USEPA, Office of Pesticide Programs, Washington, DC. 2002.
3. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Updating of the IARC Monograph, Volumes 1–42, Suppl.7. International Agency for Cancer Research, Lyon, France, 1986.
4. IARC. Occupational Exposures in Insecticide Application, and Some Pesticides. Lyon, France: International Agency for Cancer Research; 1991.
5. Repetto, R.; Baliga, S.S.; Trends and Patterns of Pesticide Use. In: Pesticides and the Immune System: Public Health Risks, 3–8. Washington, D.C.: World Resources Institute, 1996.
6. Isfort, R.J.; LeBoeuf, R.A. Application of in vitro cell transformation assays to predict the carcinogenic potential of chemicals. Mutat. Res. **1996**, *365* (1–3), 161–173.
7. Ames, B.N.; Gold, L.S. Animal cancer tests and cancer prevention. J. Natl. Cancer Inst. Monogr. **1992**, *12*, 125–132.
8. Williams, G.M.; Iatropoulos M.J.; Jeffrey, A.M. Mechanistic basis for nonlinearities and thresholds in rat liver carcinogenesis by the DNA-reactive carcinogens 2-acetylaminofluorene and diethylnitrosamine. Toxicol. Pathol. **2000**, *28* (3), 388–395.
9. Brambilla, G.; Martelli, A. Failure of the standard battery of short-term tests in detecting some rodent and human genotoxic carcinogens. Toxicology **2004**, *196* (1–2), 1–19.
10. Elespuru, R.K. Future approaches to genetic toxicology risk assessment. Mutat. Res. **1996**, *365* (1–3), 191–204.
11. Ashby, J. The value and limitations of short-term genotoxicity assays and the inadequacy of current criteria for selecting chemicals for cancer bioassays. Food. Chem. Toxicol. **1986**, *24* (6–7), 663–666.
12. Brockman, H.E.; DeMarini, D.M. Utility of short-term tests for genetic toxicity in the aftermath of the NTP's analysis of 73 chemicals. Environ. Mol. Mutagen **1988**, *11* (4), 421–435.
13. DeMarini, D.M.; Lewtas, J.; Brockman, H.E. Utility of short-term tests for genetic toxicity. Cell. Biol. Toxicol. **1989**, *5* (2), 189–200.
14. Tennant, R.W.; Margolin B.H.; Shelby, M.D.; Zeiger, E.; Haseman, J.K.; Spalding, J. et al. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science **1987**, *236* (4804), 933–941.
15. Wilbourn, J.; Haroun, L.; Heseltine, E.; Kaldor, J.; Partensky, C.; Vainio, H. Response of experimental animals to human carcinogens: an analysis based upon the IARC Monographs programme. Carcinogenesis **1986**, *7* (11), 1853–1863.
16. Gold, L.S.; Slone, T.H.; Stern, B.R.; Bernstein, L. Comparison of target organs of carcinogenicity for mutagenic and non-mutagenic chemicals. Mutat. Res. **1993**, *286* (1), 75–100.
17. McCann, J.; Choi, E.; Yamasaki, E.; Ames, B.N. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. USA **1975**, *72* (12), 5135–5139.
18. McCann, J.; Ames, B.N. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals: discussion. Proc. Natl. Acad. Sci. USA. **1976**, *73* (3), 950–954.
19. Shelby, M.D. The genetic toxicity of human carcinogens and its implications. Mutat. Res. **1988**, *204* (1), 3–15.
20. Cunningham, M.L. Role of increased DNA replication in the carcinogenic risk of nonmutagenic chemical carcinogens. Mutat. Res. **1996**, *365* (1–3), 59–69.
21. Trosko, J.E. Challenge to the simple paradigm that 'carcinogens' are 'mutagens' and to the in vitro and in vivo assays used to test the paradigm. Mutat. Res. **1997**, *373* (2), 245–249.
22. Voccia, I.; Blakley, B.; Brousseau, P.; Fournier, M. Immunotoxicity of pesticides: a review. Toxicol. Ind. Health **1999**, *15* (1–2), 119–132.
23. Pritchard, J.B.; French, J.E.; Davis, B.J.; Haseman, J.K. The role of transgenic mouse models in carcinogen identification. Environ. Health Perspect. **2003**, *111* (4), 444–54.
24. Blair, A.A.O.; Franklin, C. Carcinogenic effects of pesticides. In: SR Baker SW, editor. The Effect of Pesticides on Human Health Princeton, NJ: Princeton. Sci. 1990. p. 201–260.
25. Petrelli, G.; Siepi, G.; Miligi, L.; Vineis, P. Solvents in pesticides. Scand. J. Work Environ. Health **1993**, *19* (1), 63–65.
26. Peluso, M.; Munnia, A.; Bolognesi, C.; Parodi, S. 32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environ. Mol. Mutagen. **1998**, *31* (1), 55–59.
27. Kale, P.G.; Petty, B.T.; Jr.; Walker, S.; Ford, J.B.; Dehkordi, N.; Tarasia, S. et al. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. Environ. Mol. Mutagen. **1995**, *25* (2), 148–153.
28. Moriya, M.; Ohta, T.; Watanabe, K.; Miyazawa, T.; Kato, K.; Shirasu, Y. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. **1983**, *116* (3–4), 185–216.
29. Rank, J.; Jensen, A.G.; Skov, B.; Pedersen, L.H.; Jensen, K. Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telophase test. Mutat. Res. **1993**, *300* (1), 29–36.
30. Ecobichon, D.J. Toxic Effects of Pesticides. In: Klaassen CD, editor. Casarett & Doull's Toxicology: The Basic Science of Poisons. fifth ed., 643–689. New York: McGraw-Hill, 1996.
31. Hardell, L.; Liljegren, G.; Lindstrom, G.; Van Bavel, B.; Broman, K.; Fredikson, M.; Hagberg, H.; Nordstrom, M.; Johansson, B. Increased concentrations of chlordane in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without malignant disease. International Journal of Oncology **1996**, *9*, 1139–1242.
32. Cantor, K.P.; Strickland, P.T.; Brock, J.W.; Bush, D.; Helzlsouer, K.; Needham, L.L.; et al. Risk of non-Hodgkin's lymphoma and prediagnostic serum organochlorines: beta-hexachlorocyclohexane, chlordane/heptachlor-related compounds, dieldrin, and hexachlorobenzene. Environ. Health Perspect. **2003**, *111* (2), 179–183.
33. Dewailly, E.; Dodin, S.; Verreault, R.; Ayotte, P.; Sauve, L.; Morin, J.; et al. High organochlorine body burden in women with estrogen receptor-positive breast cancer. J. Natl. Cancer. Inst. **1994**, *86* (3), 232–234.
34. Falck, F.; Jr.; Ricci, A.; Jr.; Wolff, M.S.; Godbold, J.; Deckers, P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch. Environ. Health **1992**, *47* (2), 143–146.
35. Gammon, M.D.; Wolff, M.S.; Neugut, A.I.; Eng, S.M.; Teitelbaum, S.L.; Britton, J.A.; et al. Environmental toxins and breast cancer on Long Island. II. Organochlorine compound levels in blood. Cancer Epidemiol. Biomarkers. Prev. **2002**, *11* (8), 686–697.
36. Guttes, S.; Failing, K.; Neumann, K.; Kleinstein, J.; Georgii, S.; Brunn, H. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of

women with benign and malignant breast disease. Arch. Environ. Contam. Toxicol. **1998**, *35* (1), 140–147.
37. Hunter, D.J.; Hankinson, S.E.; Laden, F.; Colditz, G.A.; Manson, J.E.; Willett, W.C.; et al. Plasma organochlorine levels and the risk of breast cancer. N. Engl. J. Med. **1997**, *337* (18), 1253–1258.
38. Kelce, W.R.; Stone, C.R.; Laws, S.C.; Gray, L.E.; Kemppainen, J.A.; Wilson, E.M. Persistent DDT metabolite p,p′-DDE is a potent androgen receptor antagonist. Nature **1995**, *375* (6532), 581–585.
39. Lopez-Carrillo, L.; Blair, A.; Lopez-Cervantes, M.; Cebrian, M.; Rueda, C.; Reyes, R.; et al. Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: a case-control study from Mexico. Cancer Res. **1997**, *57* (17), 3728–3732.
40. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet. **1997**, *350* (9084), 1047–1059.
41. Calle, E.E.; Frumkin, H.; Henley, S.J.; Savitz, D.A.; Thun, M.J. Organochlorines and breast cancer risk. CA Cancer J. Clin. **2002**, *52* (5), 301–309.
42. Moysich, K.B.; Ambrosone, C.B.; Vena, J.E.; Shields, P.G.; Mendola, P.; Kostyniak, P.; et al. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiol. Biomarkers. Prev. **1998**, *7* (3), 181–188.
43. Wolff, M.S.; Toniolo, P.G.; Lee, E.W.; Rivera, M.; Dubin, N. Blood levels of organochlorine residues and risk of breast cancer. J. Natl. Cancer Inst. **1993**, *85* (8), 648–652.
44. Zheng, T.; Holford, T.R.; Mayne, S.T.; Ward, B.; Carter, D.; Owens, P.H.; et al. DDE and DDT in breast adipose tissue and risk of female breast cancer. Am. J. Epidemiol. **1999**, *150* (5), 453–458.
45. Bohnen, N.I.; Kurland, L.T. Brain tumor and exposure to pesticides in humans: a review of the epidemiologic data. J. Neurol. Sci. **1995**, *132* (2), 110–121.
46. Unger, M.; Olsen, J. Organochlorine compounds in the adipose tissue of deceased people with and without cancer. Environ. Res. **1980**, *23* (2), 257–263.
47. Alavanja, M.C.; Sandler, D.P.; Knott, C.; Lubin, J.H.; Tarone, R.; Blair, A. Cancer incidence in the Agricultural Health Study. Scand. J. Work Environ. Health In press.
48. Alavanja, M.C.; Samanic, C.; Dosemeci, M.; Lubin, J.; Tarone, R.; Lynch, C.F.; et al. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. Am. J. Epidemiol. **2003**, *157* (9), 800–814.
49. Blair, A.; Zahm, S.H. Cancer among farmers. Occup. Med. **1991**, *6* (3), 335–354.
50. Blair, A.; Zahm, S.H.; Pearce, N.E.; Heineman, E.F.; Fraumeni, J.F.; Jr. Clues to cancer etiology from studies of farmers. Scand. J. Work. Environ. Health **1992**, *18* (4), 209–215.
51. Buxton, J.A.; Gallagher, R.P.; Le, N.D.; Band, P.R.; Bert, J.L. Occupational risk factors for prostate cancer mortality in British Columbia, Canada. Am. J. Ind. Med. **1999**, *35* (1), 82–86.
52. Cerhan, J.R.; Cantor, K.P.; Williamson, K.; Lynch, C.F.; Torner, J.C.; Burmeister, L.F. Cancer mortality among Iowa farmers: recent results, time trends, and lifestyle factors (United States). Cancer. Causes. Control. **1998**, *9* (3), 311–319.
53. Dich, J.; Wiklund, K. Prostate cancer in pesticide applicators in Swedish agriculture. Prostate **1998**, *34* (2), 100–112.
54. Falk, R.T.; Pickle, L.W.; Fontham, E.T.; Correa, P.; Morse, A.; Chen, V.; et al. Occupation and pancreatic cancer risk in Louisiana. Am. J. Ind. Med. **1990**, *18* (5), 565–576.
55. Mills, P.K.; Yang, R. Prostate cancer risk in California farm workers. J. Occup. Environ. Med. **2003**, *45* (3), 249–258.
56. Forastiere, F.; Quercia, A.; Miceli, M.; Settimi, L.; Terenzoni, B.; Rapiti, E.; et al. Cancer among farmers in central Italy. Scand. J. Work. Environ. Health **1993**, *19* (6), 382–389.
57. Milham, S.J. Occupational Mortality in Washington State. Washington, D.C.: National Insitute of Occupational Safety and Health; 1997. Report No.: Publ. No. 96–133.
58. Alavanja, M.C.; Blair, A.; Masters, M.N. Cancer mortality in the U.S. flour industry. J. Natl. Cancer. Inst. **1990**, *82* (10), 840–848.
59. Alguacil, J.; Kauppinen, T.; Porta, M.; Partanen, T.; Malats, N.; Kogevinas, M.; et al. Risk of pancreatic cancer and occupational exposures in Spain. PANKRAS II Study Group. Ann. Occup. Hyg. **2000**, *44* (5), 391–403.
60. Figa-Talamanca, I.; Mearelli, I.; Valente, P.; Bascherini, S. Cancer mortality in a cohort of rural licensed pesticide users in the province of Rome. Int. J. Epidemiol. **1993**, *22* (4), 579–583.
61. Fryzek, J.P.; Garabrant, D.H.; Harlow, S.D.; Severson, R.K.; Gillespie, B.W.; Schenk, M.; et al. A case-control study of self-reported exposures to pesticides and pancreas cancer in southeastern Michigan. Int. J. Cancer. **1997**, *72* (1), 62–67.
62. Kauppinen, T.; Partanen, T.; Degerth, R.; Ojajarvi, A. Pancreatic cancer and occupational exposures. Epidemiology **1995**, *6* (5), 498–502.
63. Garabrant, D.H.; Held, J.; Langholz, B.; Peters, J.M.; Mack, T.M. DDT and related compounds and risk of pancreatic cancer. J. Natl. Cancer. Inst. **1992**, *84* (10), 764–771.
64. Beard, J.; Sladden, T.; Morgan, G.; Berry, G.; Brooks, L.; McMichael, A. Health impacts of pesticide exposure in a cohort of outdoor workers. Environ. Health Perspect. **2003**, *111* (5), 724–730.
65. Porta, M.; Malats, N.; Jariod, M.; Grimalt, J.O.; Rifa, J.; Carrato, A.; et al. Serum concentrations of organochlorine compounds and K-ras mutations in exocrine pancreatic cancer. PANKRAS II Study Group. Lancet. **1999**, *354* (9196), 2125–2129.
66. Slebos, R.J.; Hoppin, J.A.; Tolbert, P.E.; Holly, E.A.; Brock, J.W.; Zhang, R.H.; et al. K-ras and p53 in pancreatic cancer: association with medical history, histopathology, and environmental exposures in a population-based study. Cancer Epidemiol. Biomarkers. Prev. **2000**, *9* (11), 1223–1232.
67. Cocco, P.; Kazerouni, N.; Zahm, S.H. Cancer mortality and environmental exposure to DDE in the United States. Environ. Health Perspect. **2000**, *108* (1), 1–4.
68. Porru, S.; Placidi, D.; Carta, A.; Gelatti, U.; Ribero, M.L.; Tagger, A.; et al. Primary liver cancer and occupation in men: a case-control study in a high-incidence area in Northern Italy. Int. J. Cancer. **2001**, *94* (6), 878–883.
69. Flodstrom, S.; Hemming, H.; Warngard, L.; Ahlborg, U.G. Promotion of altered hepatic foci development in rat liver, cytochrome P450 enzyme induction and inhibition of cell-cell communication by DDT and some structurally related organohalogen pesticides. Carcinogenesis **1990**, *11* (8), 1413–1417.
70. Pesatori, A.C.; Sontag, J.M.; Lubin, J.H.; Consonni, D.; Blair, A. Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States). Cancer. Causes. Control. **1994**, *5* (4), 310–318.
71. McDuffie, H.H.; Klaassen, D.J.; Dosman, J.A. Is pesticide use related to the risk of primary lung cancer in Saskatchewan? J. Occup. Med. **1990**, *32* (10), 996–1002.
72. McDuffie, H.H.; Pahwa, P.; McLaughlin, J.R.; Spinelli, J.J.; Fincham, S.; Dosman, J.A.; et al. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health Cancer Epidemiol. Biomarkers Prev. **2001**, *10* (11), 1155–1163.
73. Shukla, Y.; Antony, M.; Mehrotra, N.K. Carcinogenic and cocarcinogenic studies with carbaryl following topical exposure in mice. Cancer. Lett. **1992**, *62* (2), 133–140.
74. Onfelt, A.; Klasterska, I. Sister-chromatid exchanges and thioguanine resistance in V79 Chinese hamster cells after treatment with the aneuploidy-inducing agent carbaryl +/− S9 mix. Mutat. Res. **1984**, *125* (2), 269–274.
75. Zheng, T.; Zahm, S.H.; Cantor, K.P.; Weisenburger, D.D.; Zhang, Y.; Blair, A. Agricultural exposure to carbamate pesticides and risk of non-Hodgkin lymphoma. J. Occup. Environ. Med. **2001**, *43* (7), 641–649.

76. Tobin, J.S. Carbofuran: a new carbamate insecticide. J. Occup. Med. **1970**, *12* (1), 16–19.
77. Hour, T.C.; Chen, L.; Lin, J.K. Comparative investigation on the mutagenicities of organophosphate, phthalimide, pyrethroid and carbamate insecticides by the Ames and lactam tests. Mutagenesis **1998**, *13* (2), 157–166.
78. Yoon, J.Y.; Oh, S.H.; Yoo, S.M.; Lee, S.J.; Lee, H.S.; Choi, S.J.; et al. N-nitrosocarbofuran, but not carbofuran, induces apoptosis and cell cycle arrest in CHL cells. Toxicology **2001**, *169* (2), 153–161.
79. Gupta, R.C. Carbofuran toxicity. J. Toxicol. Environ. Health **1994**, *43* (4), 383–418.
80. Bonner, M.R.; Lee, W.J.; Sandler, D.P.; Hoppin, J.A.; Dosemeci, M.; Alavanja, M.C. Occupational exposure to carbofuran and the incidence of cancer in the Agricultural Health Study. Environ. Health Perspect. **2005**, *113* (3), 285–289.
81. Alavanja M.C.; Dosemeci, M.; Samanic, C.; Lubin, J.; Lynch, C.F.; Barker, J.; Hoppin, J.A.; Sandler, D.P.; Coble, J.; Thomas, K.; Blair, A. Pesticides and Lung Cancer Risk in the Agricultural Health Study Cohort. American Journal of Epidemiology [In press].
82. Barnett, J.B.; Spyker-Cranmer, J.M.; Avery, D.L.; Hoberman, A.M. Immunocompetence over the lifespan of mice exposed in utero to carbofuran or diazinon: I. Changes in serum immunoglobulin concentrations. J. Environ. Pathol. Toxicol. **1980**, *4* (5–6), 53–63.
83. Fiore, M.C.; Anderson, H.A.; Hong, R.; Golubjatnikov, R.; Seiser, J.E.; Nordstrom, D.; et al. Chronic exposure to aldicarb-contaminated groundwater and human immune function. Environ. Res. **1986**, *41* (2), 633–645.
84. Smegal, D.C. Human Health RIsk Assessment-Chlorpyrifos. Washington, D.C.: USEPA; 2002.
85. Waters, M.D.; Simmon, V.F.; Mitchell, A.D.; Jorgenson, T.A.; Valencia, R. An overview of short-term tests for the mutagenic and carcinogenic potential of pesticides. J. Environ. Sci. Health B. **1980**, *15* (6), 867–906.
86. Patnaik, K.K.; Tripathy, N.K. Farm-grade chlorpyrifos (Durmet) is genotoxic in somatic and germ-line cells of Drosophila. Mutat. Res. **1992**, *279* (1), 15–20.
87. Amer, S.M.; Aly, F.A. Cytogenetic effects of pesticides. IV. Cytogenetic effects of the insecticides Gardona and Dursban. Mutat. Res. **1992**, *279* (3), 165–170.
88. Sobti, R.C.; Krishan, A.; Pfaffenberger, C.D. Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat. Res. **1982**, *102* (1), 89–102.
89. Woodruff, R.C.; Phillips, J.P.; Irwin, D. Pesticide-induced complete and partial chromosome loss in screens with repair-defective females of Drosophila melanogaster. Environ. Mutagen. **1983**, *5* (6), 835–46.
90. Lee, W.J.; Blair, A.; Hoppin, J.A.; Lubin, J.H.; Rusiecki, J.A.; Sandler, D.P.; et al. Cancer incidence among pesticide applicators exposed to chlorpyrifos in the Agricultural Health Study. J. Natl. Cancer. Inst. **2004**, *96* (23), 1781–1789.
91. Sultatos, L.G. Mammalian toxicology of organophosphorus pesticides. J. Toxicol. Environ. Health **1994**, *43* (3), 271–189.
92. Dick, R.B.; Steenland, K.; Krieg, E.F.; Hines, C.J. Evaluation of acute sensory–motor effects and test sensitivity using termiticide workers exposed to chlorpyrifos. Neurotoxicol. Teratol. **2001**, *23* (4), 381–393.
93. Roy, T.S.; Andrews, J.E.; Seidler, F.J.; Slotkin, T.A. Chlorpyrifos elicits mitotic abnormalities and apoptosis in neuroepithelium of cultured rat embryos. Teratology **1998**, *58* (2), 62–68.
94. Blakley, B.R.; Yole, M.J.; Brousseau, P.; Boermans, H.; Fournier, M. Effect of chlorpyrifos on immune function in rats. Vet. Hum. Toxicol. **1999**, *41* (3), 140–144.
95. Bagchi, D.; Bagchi, M.; Hassoun, E.A.; Stohs, S.J. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology **1995**, *104* (1–3), 129–140.
96. Thrasher, J.D.; Heuser, G.; Broughton, A. Immunological abnormalities in humans chronically exposed to chlorpyrifos. Arch. Environ. Health **2002**, *57* (3), 181–187.
97. Bebe, F.N.; Panemangalore, M. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. J. Environ. Sci. Health B **2003**, *38* (3), 349–363.
98. Charles, J.M.; Cunny, H.C.; Wilson, R.D.; Bus, J.S.; Lawlor, T.E.; Cifone, M.A.; et al. Ames assays and unscheduled DNA synthesis assays on 2, 4-dichlorophenoxyacetic acid and its derivatives. Mutat. Res. **1999**, *444* (1), 207–16.
99. Charles, J.M.; Cunny, H.C.; Wilson, R.D.; Ivett, J.L.; Murli, H.; Bus, J.S.; et al. In vivo micronucleus assays on 2,4-dichlorophenoxyacetic acid and its derivatives. Mutat. Res. **1999**, *444* (1), 227–234.
100. Charles, J.M.; Leeming, N.M. Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxybutyric acid in rodents. Toxicol. Sci. **1998**, *46* (1), 21–30.
101. Becher, H.; Flesch-Janys, D.; Kauppinen, T.; Kogevinas, M.; Steindorf, K.; Manz, A.; et al. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer. Causes. Control. **1996**, *7* (3), 312–321.
102. Hooiveld, M.; Heederik, D.J.; Kogevinas, M.; Boffetta, P.; Needham, L.L.; Patterson, D.G.; Jr.; et al. Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. Am. J. Epidemiol. **1998**, *147* (9), 891–901.
103. Lynge, E. Cancer incidence in Danish phenoxy herbicide workers, 1947–1993. Environ. Health Perspect. **1998**, *106 Suppl 2*, 683–688.
104. Asp, S.; Riihimaki, V.; Hernberg, S.; Pukkala, E. Mortality and cancer morbidity of Finnish chlorophenoxy herbicide applicators: an 18-year prospective follow-up. Am. J. Ind. Med. **1994**, *26* (2), 243–253.
105. Morrison, H.I.; Semenciw, R.M.; Wilkins, K.; Mao, Y.; Wigle, D.T. Non-Hodgkin's lymphoma and agricultural practices in the prairie provinces of Canada. Scand. J. Work Environ. Health **1994**, *20* (1), 42–47.
106. Thorn, A.; Gustavsson, P.; Sadigh, J.; Westerlund-Hannestrand, B.; Hogstedt, C. Mortality and cancer incidence among Swedish lumberjacks exposed to phenoxy herbicides. Occup. Environ. Med. **2000**, *57* (10), 718–720.
107. Zahm, S.H. Mortality study of pesticide applicators and other employees of a lawn care service company. J. Occup. Environ. Med. **1997**, *39* (11), 1055–1067.
108. Hardell, L.; Eriksson, M. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer **1999**, *85* (6), 1353–1360.
109. Hardell, L.; Eriksson, M.; Nordstrom, M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk. Lymphoma. **2002**, *43* (5), 1043–1049.
110. Kogevinas, M.; Kauppinen, T.; Winkelmann, R.; Becher, H.; Bertazzi, P.A.; Bueno-de-Mesquita, H.B.; et al. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. Epidemiology **1995**, *6* (4), 396–402.
111. Persson, B.; Fredriksson, M.; Olsen, K.; Boeryd, B.; Axelson, O. Some occupational exposures as risk factors for malignant lymphomas. Cancer **1993**, *72* (5), 1773–1778.
112. Wetzel, L.T.; Luempert, L.G.; 3rd, Breckenridge, C.B.; Tisdel, M.O.; Stevens, J.T.; Thakur, A.K.; et al. Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. J. Toxicol. Environ. Health **1994**, *43* (2), 169–182.
113. Stevens, J.T.; Breckenridge, C.B.; Wetzel, L. A risk characterization for atrazine: oncogenicity profile. J. Toxicol. Environ. Health A **1999**, *56* (2), 69–109.
114. Pinter, A.; Torok, G.; Borzsonyi, M.; Surjan, A.; Csik, M.; Kelecsenyi, Z.; et al. Long-term carcinogenicity bioassay of the herbicide atrazine in F344 rats. Neoplasma **1990**, *37* (5), 533–544.

115. Donna, A.; Betta, P.G.; Gagliardi, F.; Ghiazza, G.F.; Gallareto, M.; Gabutto, V. Preliminary experimental contribution to the study of possible carcinogenic activity of two herbicides containing atrazine-simazine and trifuralin as active principles. Pathologica **1981**, *73* (1027), 707–721.
116. Donna, A.; Betta, P.G.; Robutti, F.; Bellingeri, D. Carcinogenicity testing of atrazine: preliminary report on a 13-month study on male Swiss albino mice treated by intraperitoneal administration. G. Ital. Med. Lav. **1986**, *8* (3–4), 119–121.
117. IARC. Summaries and Evaluations. Atrazine. 1999. Lyon, France: World Health Oganization.
118. EPA U. Revised atrazine interim reregistration eligibility decision (IRED). Washington, D.C.: USEPA; 2003.
119. MacLennan, P.A.; Delzell, E.; Sathiakumar, N.; Myers, S.L. Mortality among triazine herbicide manufacturing workers. J. Toxicol. Environ. Health A. **2003**, *66* (6), 501–517.
120. Hoar, S.K.; Blair, A.; Holmes, F.F.; Boysen, C.D.; Robel, R.J.; Hoover, R.; et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Jama. **1986**, *256* (9), 1141–1147.
121. Brown, L.M.; Blair, A.; Gibson, R.; Everett, G.D.; Cantor, K.P.; Schuman, L.M.; et al. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer. Res. **1990**, *50* (20), 6585–6591.
122. Burmeister, L.F. Cancer in Iowa farmers: recent results. Am. J. Ind. Med. **1990**, *18* (3), 295–301.
123. Hoar, S.K.; Blair, A.; Holmes, F.F.; Boysen, C.; Robel, R.J. Herbicides and colon cancer. Lancet. **1985**, *1* (8440), 1277–1278.
124. De Roos, A.J.; Zahm, S.H.; Cantor, K.P.; Weisenburger, D.D.; Holmes, F.F.; Burmeister, L.F.; et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occup. Environ. Med. **2003**, *60* (9), E11.
125. Donna, A.; Crosignani, P.; Robutti, F.; Betta, P.G.; Bocca, R.; Mariani, N.; et al. Triazine herbicides and ovarian epithelial neoplasms. Scand. J. Work. Environ. Health **1989**, *15* (1), 47–53.
126. Rusiecki, J.A.; De Roos, A.; Lee, W.J.; Dosemeci, M.; Lubin, J.H.; Hoppin, J.A.; et al. Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. J. Natl. Cancer. Inst. **2004**, *96* (18), 1375–1382.
127. Williams, G.M.; Kroes, R.; Munro, I.C. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul. Toxicol. Pharmacol. **2000**, *31* (2 Pt 1), 117–165.
128. Bolognesi, C.; Bonatti, S.; Degan, P.; Peluso, M.; Rabboni, R.; Abbondandolo, A. Genotoxic activity of glyphosate and its techinal formulation. Journal of Agricultural and food chemistry **1997**, *45*, 1957–1962.
129. Vigfusson, N.V.; Vyse, E.R. The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. Mutat. Res. **1980**, *79* (1), 53–57.
130. De Roos, A.; Blair, A.; Rusiecki, J.; Hoppin, J.A.; Svec, M.; Dosemeci, M.; Sandler, D.P.; Alavanja, M.C.R. Cancer Incidence Among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study. Environ Health Perspect In press.
131. Eastmond DaB, S. Genetic Toxicology of Pesticides. In: Krieger R, editor. Handbook of Pesticide Toxicology 2nd ed. San Diego: Academic Press; 2001. p. 747–767.
132. Kavlock, R. Pesticides as Endocrine-Disrupting Chemicals. In: Krieger R, editor. Handbook of Pesticide Toxicology 2nd ed., 727–746. San Diego: Academic Press, 2001.
133. Rodgers, K. Immunotoxicity of Pesticides. In: Krieger RI, editor. Handbook of Pesticide Toxicology 2nd ed., 769–782. San Diego: Academic Press, 2001.
134. Garry, V.F.; Griffith, J.; Danzl, T.J.; Nelson, R.L.; Whorton, E.B.; Krueger, L.A.; et al. Human genotoxicity: pesticide applicators and phosphine. Science 1989;246 (4927), 251–5.
135. Garry, V.F.; Tarone, R.E.; Long, L.; Griffith, J.; Kelly, J.T.; Burroughs, B. Pesticide appliers with mixed pesticide exposure: G-banded analysis and possible relationship to non-Hodgkin's lymphoma. Cancer Epidemiol. Biomarkers Prev. **1996**, *5* (1), 11–16.
136. Brogger, A.; Hagmar, L.; Hansteen, I.L.; Heim, S.; Hogstedt, B.; Knudsen, L.; et al. An inter-Nordic prospective study on cytogenetic endpoints and cancer risk. Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Genet. Cytogenet. **1990**, *45* (1), 85–92.
137. Hagmar, L.; Brogger, A.; Hansteen, I.L.; Heim, S.; Hogstedt, B.; Knudsen, L.; et al. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer. Res. **1994**, *54* (11), 2919–2922.
138. Bonassi, S.; Abbondandolo, A.; Camurri, L.; Dal Pra, L.; De Ferrari, M.; Degrassi, F.; et al. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer. Genet. Cytogenet. **1995**, *79* (2), 133–135.
139. Hagmar, L.; Stromberg, U.; Bonassi, S.; Hansteen, I.L.; Knudsen, L.E.; Lindholm, C.; et al. Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer. Res. **2004**, *64* (6), 2258–2263.
140. Hagmar, L.; Bonassi, S.; Stromberg, U.; Brogger, A.; Knudsen, L.E.; Norppa, H.; et al. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer. Res. **1998**, *58* (18), 4117–4121.